In silico prioritisation of endocrine active substances (EAS) and their *in vitro* validation

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Introduction

In silico molecular docking can be a cheap and fast strategy to estimate the binding free energies, and consequently the dissociation constants, for a set of compounds with respect to their putative targets. Interesting targets for EAS are the ligand binding domains (LBD) of the human nuclear receptors for the sex hormones, i.e. the estrogen, androgen, progesterone, and of the (gluco)corticoid receptor[1]. The Horizon 2020 project EuroMix (http://euromixproject.eu) aims to establish and disseminate new, efficient and validated strategies for the risk assessment of mixtures, while limiting the use of test animals. The present work deals with a part of EuroMix that is intended to set up a testing approach for mixtures of endocrine active chemicals, focusing on estrogenic effects.

For that purpose, a combined Adverse Outcome Pathway (AOP) was constructed, including Molecular Initiating Events, Key Events, and Adverse Outcome (reproductive dysfunction). Using this combined AOP as framework, cognate *in silico* and *in vitro* tools as well as the *in vivo* confirmation studies were selected, i.e. *in silico*: h-ER and h-AR docking; *in vitro*: cell-based ER and AR transcriptional activation bioassays and the H295R steroidogenesis assay; and *in vivo*: the Fish Sexual Development Test (FSDT, OECD Test No. 234) and a rat study, examining in (male) offspring a number of parameters, such as anogenital distance, cryptorchidism, and nipple retention.

Molecular docking of EUROMIX database on h-Estrogen Receptor alpha (h-ERα) LBD

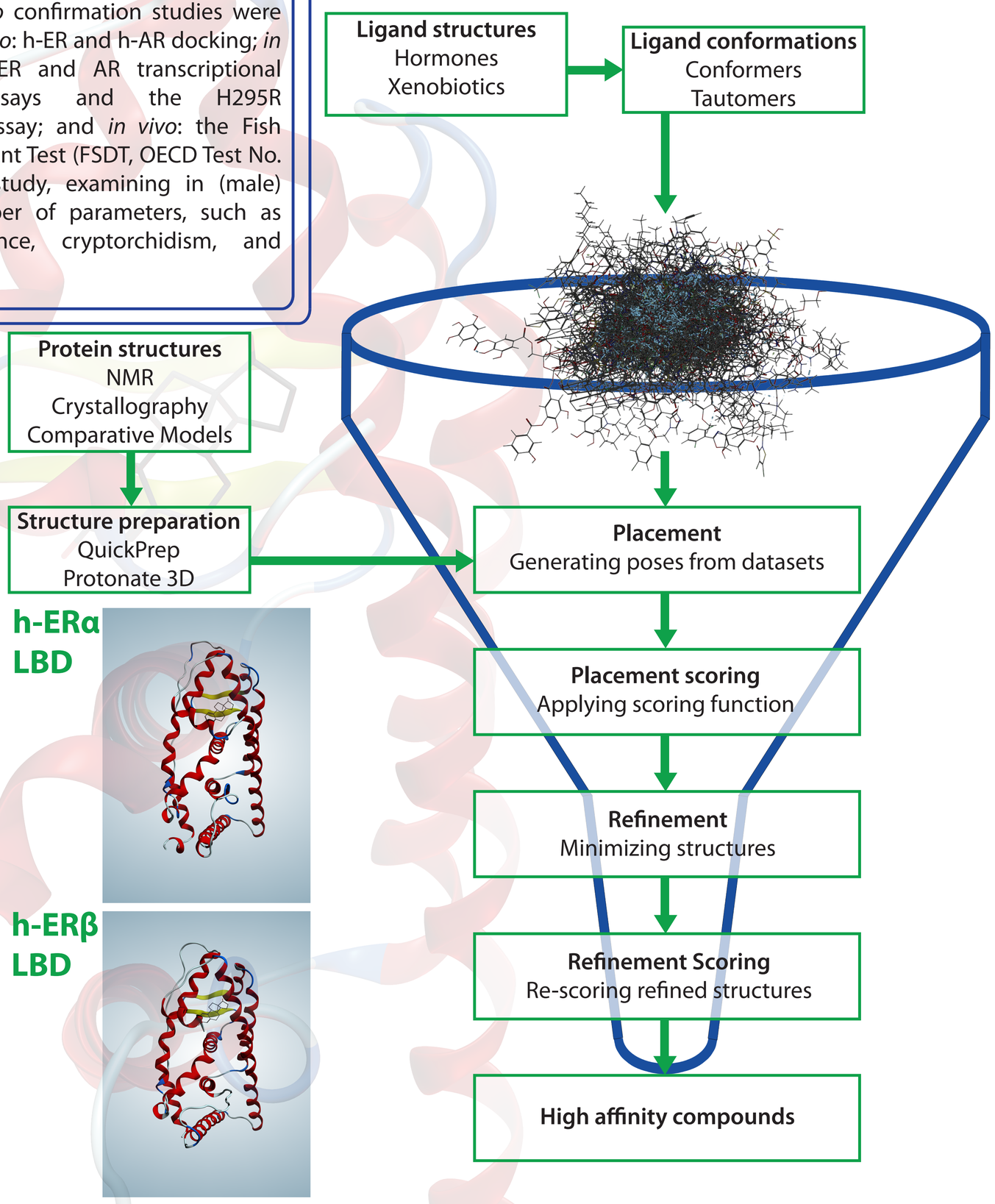
Compound	Binding free energy (kcal/mol)	Dataset
Ethanol, 2-[2-(4-nonylphenoxy)ethoxy]-	-9.2	Easis.sdf
Azoxystrobin	-8.4	PPP.sdf
Zearalenone	-8.1	Mycotoxin.sdf
17 beta-estradiol	-8.1	Natural ligands

Molecular docking of EUROMIX database on h-Estrogen Receptor beta (h-ERβ) LBD

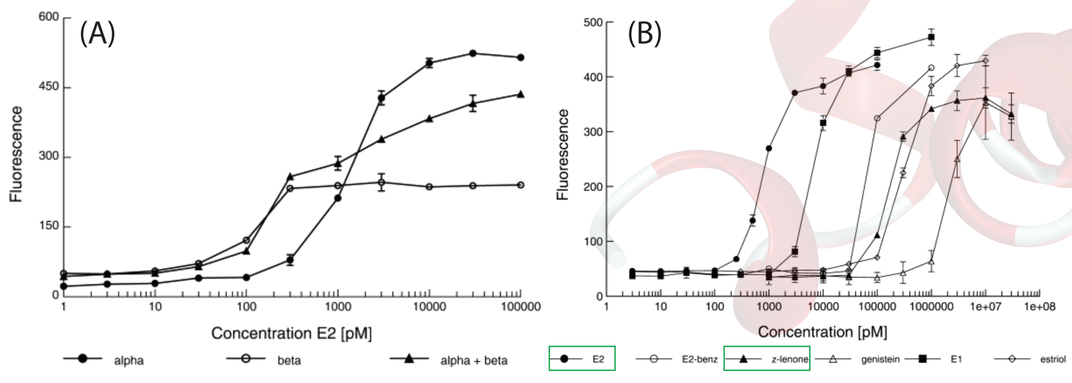
Compound	Binding free energy (kcal/mol)	Dataset
Endosulfan	-9.9	PPP.sdf
Ethanol, 2-[2-(4-nonylphenoxy)ethoxy]-	-8.6	Easis.sdf
Heptachlor	-8.5	PPP.sdf
Spirodiclofen	-8.1	PPP.sdf
Dodine	-8.0	PPP.sdf
Cyclotetrasiloxane	-8.0	Easis.sdf
Lycopsamine	-7.9	Alkaloid.sdf
Malathion	-7.9	PPP.sdf
Zearalenone	-7.9	Mycotoxin.sdf
Hexythiazox	-7.9	PPP.sdf
Penthiopyrad	-7.9	PPP.sdf
Ranitidine hydrochloride	-7.8	HepNegData.sdf
17 beta-estradiol	-7.7	Natural ligands

In silico prioritisation of EAS

The first prioritization pipeline is aimed at computing the affinity of all tested compounds with respect the selected targets. Affinity can be expressed as binding free energy or dissociation constant. No intrinsic activity estimation is provided by this pipeline, making it impossible to classify the tested compounds as agonists or antagonists, without any further evaluation.



In vitro validation[2]



(A) *Response of the yeast ERα cytosensor, ERβ cytosensor and the yeast cytosensor that express both the ERα and the ERβ after exposures for 4h to 17 β-estradiol.* (B) *Response of the yeast ERα cytosensor to different substances after 4h exposure period: 17 β-estradiol (E2), β-estradiol 3-benzoate (E2-benz), zearalenone (z-lenone), genistein, estrone (E1) or estriol.*

Conclusions

Our preliminary work has been focused just on compounds with affinities higher than the natural hormone ones. Here we reported a comparison between computational and experimental data on the estrogenic system, but a similar approach can be followed for the other investigated targets.

So far, *in silico* and *in vitro* testing of endocrine active reference chemicals show a good correlation between the *in silico* determined binding energies and the *in vitro* measured hormonal activities.

References

[1] T. F.H. Bovee et al., *A new highly androgen specific yeast biosensor, enabling optimisation of (Q)SAR model approaches*, J Steroid Biochem Mol Biol. 108 (2008) 121–131.

[2] T. F.H. Bovee at al., *Rapid yeast estrogen bioassay stably expressing human estrogen receptors alpha and beta, and green fluorescent protein: a comparison of different compounds with booth receptor types*, J Steroid Biochem Mol Biol. 2004 Jul;91(3):99-109

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